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Sterility and Secondary Sexual Characters of Triploid Gnathopogon elongatus caerulescens*

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Summary : Triploids of Gnathopogon elongatus caerulescens (Cyplinid fish) were induced by the cold shock treatment of the fertilized eggs. From the eggs cooled in a 4°C iced water for 60 min starting 5 min after insemination were induced triploids at the incidence of 95%. Observations of gonad and secondary sexual character(pearl organ) were made during the spawing season for this fish. The triploid ovaries were identical in external appearance with the diploid ovaries. Some of their ovarian eggs reached the migratory nucleus stage, but showed no meiosis. The triploid testes were inferior in external appearance, but produced sperms. The head of their pserms were generally large-sized compard with the diploid sperms.

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*Translator's note: "Biwa gudgeon" in the present translation.

Technically, it is easy to induce artificial triploidy in fish. As it is obtained at a high frequency (proportion among fish produced: 80-100)[2,4,5], as virtually no genetic trouble is encountered during the formation stage and as the hatching and production rates of fry are high, it is in fish breeding that artificial polyploidy seems to be most easily induced. Various effects concerning fish culture are expected from the sterility and from the drop in appearance of secondary sexual characters supposed to occur along with the change to sterility produced in triploids. Studies made so far about artificial triploid fish have not presented in-depth views about these characteristics and one would like to see more investigations about the characteristics and their advantages with regard to the use of triploids.

In a series of studies about artificial polyploid fish, we produced Biwa gudgeon triploids and were able to watch in detail the development of their gonads and the appearance of the secondary sexual characters (pearl organs). In the present report we will rely on our results to discuss the induction conditions based on cold treatment of triploids and the practical usefulness of bred sterility.

Material and method

We performed artificial fertilization by the wet induction method, using as parents Biwa gudgeons taken from the Biwa lake (we used 2 females and 4 males on May 20, 1982). We allowed the eggs to get attached to several pieces of stainless metallic mesh (10 x 10 cm, 0.25 mm openings) and performed under these conditions the cold

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treatment as well as the following controls. For the cold treatment, we dropped one portion of the eggs for 30 min in 4°C ice water 5 minutes after insemination and the other portion was kept in the same ice water for 60 min. Former and posterior treatments were carried out at 22°C. Twenty months after hatching (in the meantime the fish had been reared indoors in 45 x 90 x 45 cm glass tanks), there were among the test fish some who could yield sperm through artificial stripping. The karyotype analysis of the test fish and the observation of the gonads were performed from that moment on for two months, i.e. when these individuals seemed to be most mature. The chromosomes samples were prepared through the regular air-drying method (50 γ /mL Korusemido* solution was injected peritoneally at the rate of 0.01 mL per 1 g of fish body weight; the renal tissue were excised after 3-4 hours, the isolated cells were given an hypotonic treatment with 0.05 MKCl for 30-50 min and fixed while changing three times the Carnoy's fluid, and stained with giemsa). The histological samples of the gonads were fixed in formalin and hardened by the paraffin imbedding method (10 μ sections, Karatchi* H. EY staining) while the spermatozoid samples were fixed in a 1% glutaraldehyde - 0.1 M phosphoric acid buffer solution, dehydrated with an acetonic series, prepared through critical point drying followed by scanning electron microscope observation. The characteristics of the various forms of the triploids were compared with those of the diploids produced in the testing area.

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*Translator's note: As transliterated; probably English term of uncertain rendering.

Results and discussion p. 38

1. Triploid inducement conditions

On the base of the results obtained through studies on cyprinids, the low temperature treatment was commenced 5 min after insemination[4, 5]. This starting time is extremely effective in inducing triploidy in the present fish species (Table 1). This suggested that the low-temperature treatment works effectively from the middle period to the beginning of the last period of the 2nd meiosis. A 30-min cold treatment had almost no adverse effect on the formation of the eggs and the triploid appearance frequency remained around 50%. On the other hand, the 60-min treatment gives a hatching rate of about 20% which is lower than with the former treatment but the appearance frequency of triploids reached 94% (Table 1). The 60-min treatment is markedly bad for the egg formation (hatching rate below 20%) of carps[5] and crucians (unpublished) and the 30-min treatment seems more appropriate. On this point, it is shown that the correct treatment for triploid production differs even among fish belonging to the same cyprinid family.

All fish on which we performed karyotype analysis in the present study (48 individuals) were diploid (2 n = 50, composition of 17 metacentric-submetacentric filiform chromosomes pairs and of 8 subtelocentric-acrocentric pairs of filiform chromosomes) or triploid (Fig. 1). In the studies conducted so far on the triploid induction among fish, not only diploids and triploids but haploids, mosaics[3] and tetraploids as well were simultaneously obtained. The appearance of haploids is seen when the physical stimulation toward fertilization

ends in failure (the secondary polar bodies are released) and when either the spermatozoid nuclei or the ovarian nuclei (n) lose their genetic strength because of these treatments. p. 39

It is possible that mosaics are produced when the nuclei of the spermatozoids and of the eggs are damaged through physical handling and when the fusion or fission of the nuclei is abnormal. However, practically no normal embryonic formation can be expected from eggs having these elements, and it seems that even when hatching takes place, the larval fish reaching adulthood are extremely few. As for triploid induction we want to think first that the induction mechanisms regarding both the triploids and the concurrently generated tetraploids are cytologically entirely different. In the present study we were unable to observe a single haploid or mosaic but this may be due to the fact that the karyotype analysis was based on fish having reached maturity.

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2. The sterility of triploids

A macroscopic examination reveals that the testes of triploids are bilaterally symmetrical, that they show a uniform milky white color, and no abnormality was noted in the external shape. The development, however, varied seriously among the individuals and was on the whole inferior to that of the diploids. Fig. 2A shows the testes (medium development) of a triploid. In the present study the sex rate shows more males among both diploids and triploids. Among 34 diploid individuals, 8 were female. Observations limited to these individuals reveal that the ovaries are always symmetrically distributed to the left and right and fairly developed in outward appearance. They were

full of large eggs with egg yolk accumulation and the entire abdominal cavity was filled up (Fig. 2B).

We prepared histological samples by excising tissues from the center and both extremities of the gonads. The pictures show that in the testes of diploids the formation of spermatozoids progresses simultaneously over the entirety of the testes, that the utricule or leaflet disintegrated and that the inside of the testes was packed with spermatozoids (Fig. 3A).

In the testes of the triploids, too, we noted an overall multiplication of the reproductive cells but the inside of the leaflet was dominated by primary and secondary spermatocytes, and the formation of spermatids and spermatozoids was localized (Fig. 3B). Numerous ovaries of diploids were constituted of primary, secondary oocytes and already freed mature eggs. On the other hand, most ovary eggs of the triploids had reached the yolk cell stage or the yolk sphere stage (Fig. 3E). Part of the eggs was at the germ cell migration stage (Fig. 3F) but we saw no individuals having released polar bodies. A macroscopic histological observation revealed that all the Biwa gudgeon diploids examined had one of the gonads, that is either testes or an ovary, but that none had both.

As we saw first, spermatozoids are formed in the testes of the triploids. To examine the form and vitality of these spermatozoids, we pressed the abdomen and squeezed out the sperm. We obtained sperm from 24 of the 26 triploids examined. We saw various morphological abnormalities among the spermatozoids of the triploids but many had a head, a middle segment, a tail part. The heads of the spermatozoids of the triploids are spherical like those of diploids. The sizes

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(diameter) are not uniform and most were larger than in diploids (diploids: 1.6-1.7 μm; triploids: 1.7-2.1 μm) (Fig. 3C, D). These spermatozoids grew in vigor when coming in contact with water, and moving forward performed pendulum motions.

Regarding the question of how to use diploid sterility in the breeding of fish, the answers differ according to the degree of sterility. Sterility may be divided into several degree levels. We divided as follows: 1) the reproductive cells show incomplete reproduction (degeneration of the gonads), 2) the reproductive cells reach the growth period but meiosis does not take place (no possible gamete formation), 3) there is gamete formation but there are morphological and functional troubles (fertilization not possible; abnormal embryo generation). About the utility of the various degrees, the opinions are as follows. Degree 1): since the gonads do not develop, the components used normally for their formation, are used for the formation of the other parts of the body and this is actually helpful toward increasig and developing the edible parts. Degree 2): these fish which do not ovulate in spite of having developed gonads might maintain for a long time the so-called seed condition (fish having developed testes). Degree 3): the spermatozoids of the triploids seem to have individual varied chromosomal structures. The eggs fertilized by these chromosomes hardly yield a growth of normal individuals. However, spermatozoids having a chromosomal structure might produce normal egg development and yield heteroploids having a practical value. Furthermore, unlike those fish which die after spawning or spermiating (for example, the salmonids), the present 1), 2) categories fish might survive after the spawning season and there seems to be no risk that

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they could disturb the ecological system as transplanted fish (through multiplication, crossing).

Among triploid Biwa gudgeons, the development of the testes differed from that of the ovaries. The former was at the 2), 3) levels and the latter at level 2). So we see that the use of triploids will in this respect be different for females and males.

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3. Secondary characters of triploids

The sexual hormones of fish originate from the gonads. The male hormones seem to be produced by the Leydig's cells and the female hormones by the follicles and capsule cells. As secondary characters are controlled by the hormones their appearance in triploids will differ according to the level of development of the gonads. It seems that if there is gonad degeneration, they will be weak, and that they will be closely similar to those of diploids if the glands are developed. If triploids exhibit the former condition (degeneration) the following effect may be expected in the course of breeding: 1) fish whose commercial value drops because of the appearance of nuptial colors or of the pearl organs can only be used as commercial product, 2) they may lose those characteristics which are disadvantageous from the viewpoint of rearing (aggressivity during oviposition period).

During the oviposition season, the Biwa gudgeons display pearl organs as secondary characters. They can be seen over the entire body of both males and females. They are also seen on the body of both female and male triploid Biwa gudgeons. The level of appearance is almost similar to that noted among diploids. As we saw, the gonads of

triploid Biwa gudgeons show the levels 2), 3) with regard to development and the outward appearance was similar to that of the diploids. These results corroborate the former estimation about sterility and the appearance of secondary characters and it is suggested that the attempt at inhibiting the appearance of secondary characters through conversion to triploidy can be used only for fish species having degenerative gonads.

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Table 1 Hatching rate and fr[illegible]y of the triploid occurrence obtained by subjecting the egg to cold shock of 4°C at 5 minutes after insemination

Experiment	Duration of treatment (hr)	Total no of eggs treated	Hatched No	Hatched %	No of analysable	Diploid No	Diploid %	Triploid No	Triploid %
Lot I	1.2	116	98	84.5	28	13	46.4	15	53.6
Lot II	1	121	75	62.0	20	1	5.0	19	95.0

Fig.1. Metaphase spread (A) and karyotype (B) of triploid *Gnathopogon elongatus caerulescens*. Triploids has 2n=3x= 75 chromosomes. These chromosomes are classified into 25 groups of 3 chromosomes each : 17 groups of metacentric-submetacentrics and 8 groups of subtelocentric-acrocentrics.

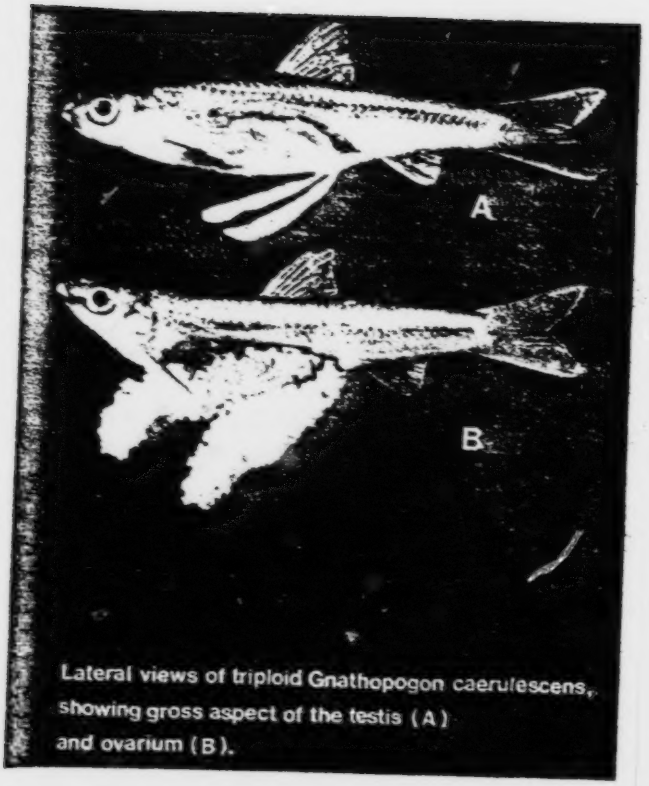

Fig.2. External appearances of the testis (A) and ovary (B) of two-years-old triploid fish. The testis is an instance with moderate development, sperm of which could be artificially stripped, and the ovary is a most well developed one.

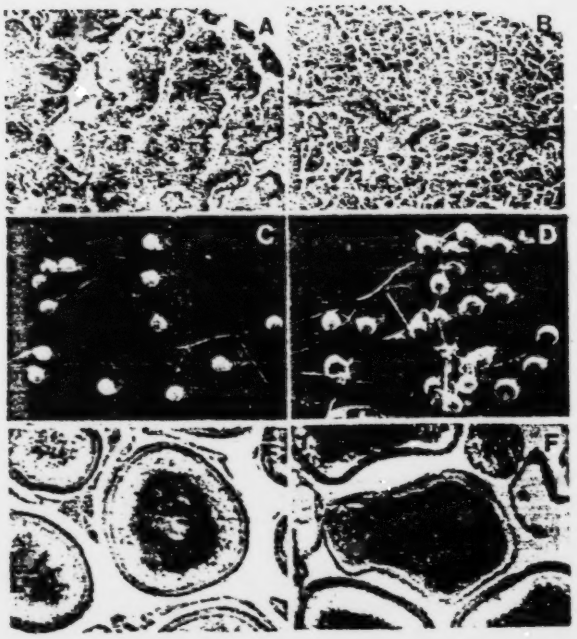

Fig.3. Histological pictures of the diploid and triploid gonads

(A) Diploid testis : The testis is filled with sperms.

(B) Triploid testis : Spermes are observed in few lobules.

(C) Diploid sperms

(D) Triploid sperms (of the same magnification as C) : The head of sperms are large-sized compered with the diploid sperms, and are uneven.

(E,F) Triploid ovarian eggs : F is at the migratory nucleus stage, where reach a few of the eggs.

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